Transcribing and translating mCherry without cells

1. Set up PCR for negative control, positive control (luciferase), and mCherry.

    a. 2 microliter DNA template
    b. 5 microliter Amino Acid mixture (mix equal volumes of two amino acid mixture minus methionine and amino acid mixture minus cysteine; mix gently before use)
    c. 20 microliters S30 premix without amino acid (mix before use)
    d. 15 microliters S30 extract, circular (mix gently before use)
    e. total volume: 50 microliters (add nuclease free to get final volume)

2. Vortexed gently and centrifuged for 5s.

3. Incubated 37C for 1 h.

4. Stopped rxn by placing in ice bath for 5 minutes.

5. Added 100 microliter of luciferase in A1, A2, A3, A4.

    a. Negative Control B1
    b. mCherry C1

6. Analyzed results using Tecan infinite m1000 plate reader at 37 C.

    a. Shaking 5 s, Amplitude 4 mm
    b. Luminescence:

        i. Attenuation: Automatic

        ii. Integration time: 10000 ms

        iii. Settle time: 1000 ms

        iv. Label name: Luciferase

    c. Fluorescence Intensity:

        i. Excitation = 587 nm

        ii. Emission = 610 nm

        iii.Gain: Calculated from well C1 (mCherry)

        iv.Flashes: Mode 1 (400 Hz) 50

        v. Z-Position: Manual 20000 micrometer

        vi. Label Name: mCherry

7. For mCherry 55 microliters dH2O to wells, 45 microliters of mixture + assay reagent

    a. For luciferase: 50 microliters dH2O to wells,

8. Added 50 microliters of assay reagent to PCR tubes.

9. Added 100 microliters from PCR tube to well.
10. Used plate reader.